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AN INVASIVE CRAB IN THE SOUTH ATLANTIC BIGHT: FRIEND OR FOE?

Final Report
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ABSTRACT

Biological invasions threaten natural communities and are major forces in determining the structure of ecosystems. Recently, the green porcelain crab, *Petrolisthes armatus*, invaded oyster reefs of the South Atlantic Bight at densities up to several thousand m^{-2}. Quarterly monitoring in two Georgian estuaries showed that crab densities increased from the upper to lower regions of the estuaries and were higher in the low versus high intertidal. In warmer months 20 – 90 % of mature females were gravid, recruits were dense, and population levels elevated, but in colder months densities decreased by 64 – 99.7 %. Sex ratios varied around 1 : 1, and correlations of native versus exotic crabs were mostly positive; never significantly negative, indicating no strong negative interactions. Mesocosm experimentation in which oyster communities were seeded with the crab suggested that *P. armatus* can potentially cause significant changes in reefs. Native species recruitment did not change in the presence of *P. armatus*, but more species-specific effects appeared. The crab depressed oyster growth, moderated seasonal fluctuations in microalgal biomass on oyster surfaces, and facilitated the growth of large macrophytes. Although these results indicate what may be affected by the invasion, the long-term, large-scale ecological effects have yet to be seen.

Key Words: *Petrolisthes armatus*, biological invasion, oyster reefs, South Atlantic Bight, monitoring, population dynamics, mesocosms

INTRODUCTION

Biological invasions can threaten the integrity of natural communities and are a major force in determining the structure of ecosystems (Mooney and Drake 1986, Drake *et al.* 1989, Simberloff *et al.* 1997). The characteristics and ecological impacts of exotic species provide insights into understanding community assembly (e.g., predator-prey interactions, community complexity and stability, competitive exclusion) and global change (e.g., climate change, habitat fragmentation, declining biodiversity) (Lodge 1993). Although much of conservation focuses on the welfare of single species, today's environmental problems require that biological invasions be considered in light of interactive processes and ecosystem function (Meffe and Carroll 1997).

Most studies of invasions have focused on terrestrial and freshwater habitats in which successful invaders have had catastrophic impacts on native species (Carlton and Geller 1993). Despite many known cases of successful marine invasions, they have been studied far less but are of such magnitudes that they may produce profound ecological changes in the ocean (Hedgpeth 1993, Carlton and Geller 1993, Cohen and Carlton 1998). Biotic homogenization within and among continents is rivaled by the mixing of organisms among the oceans (Mooney and Cleland 2001).

Oyster reefs of the South Atlantic Bight (SAB), U.S.A. are the major hard-substrate communities in these coastal areas, and they rarely have been affected by invasions of invertebrate macro-fauna known to be succeeding in ports and estuaries within the United States (Ruiz *et al.* 1997, Cohen and Carlton 1998, Ruiz *et al.* 1999). However, in 1995

the green porcelain crab, *Petrolisthes armatus*, colonized oyster reefs of the SAB and rapidly increased from 1 individual m^{-2} in 1995 to over 16,000 m^{-2} by August 1999 (South Carolina Department of Natural Resources, http://water.dnr.state.sc.us/marine/mrri/shellfish/petro.htm). In its native range including the eastern Pacific (Gulf of California to Peru), western Atlantic (Bermuda, Gulf of Mexico, Caribbean, and Brazil), and western Africa (Oliveira and Masunari 1995), the crab is found at densities of only $\leq$ 305 individuals m^{-2} (highest documented densities, Oliveira and Masunari 1995).

In its new range, *P. armatus* is found primarily in oyster reef habitats of Georgia and South Carolina. Although this crab has been found across a wide area of the tropical Atlantic and Pacific from rocky shores (Oliveira and Masunari 1995), sand reefs built by sabellid worms (Micheletti-Flores and Negreiros-Fransozo 1999), and even mangrove prop roots (Sheridan 1992), very little is known about the crab's distribution, habitat preference, seasonal fluctuations, population structure, and food web or community effects (but see Sheridan 1992 for density fluctuations in Rookery Bay, Florida and Oliveira and Masunari 1995 and Micheletti-Flores and Negreiros-Fransozo 1999 for population dynamics in southern Brazil). Therefore, through our work we assessed the population dynamics of the crab in a large-scale monitoring program and determined the forces behind the success of the invasion and the potential impacts of the crab on oyster reef communities in mesocosm and field studies. For the purposes of this report we focus on the monitoring and mesocosm work discussed and detailed in the original fellowship proposal. A more complete account of further field and laboratory experimentation will follow in the completed doctoral dissertation.

MATERIALS AND METHODS

Monitoring

Study Sites

Monitoring of *Petrolisthes armatus*, as well as associated oyster reef crabs, was performed within two estuaries of coastal Georgia. The first estuary at Sapelo Island, GA is part of the National Oceanic and Atmospheric Administration's National Estuarine Research Reserve System (Figure 1) and incorporates the Duplin River (~ 12.5 km). The Duplin River is a river in name only because it does not have a significant freshwater source, therefore it can be more appropriately described as an elongated tidal embayment (Ragotzkie and Bryson 1955, Chalmers 1997). The transport of materials in the river is primarily diffusive except when there is heavy rainfall at low tide which can nearly replace the water in the upper regions (Chalmers 1997). The Duplin River has three tidal prisms along its length (Ragotzkie and Bryson 1955). Strong tidal currents at the mouth of the river and the lack of freshwater input at the head of the river maintain a well-mixed, vertically homogeneous water column within each tidal prism, but the overall impact is little advective transport of water and materials (Ragotzkie and Bryson 1955, Bahr and Lanier 1981, Chalmers 1997). Thus, the water of the upper regions of the river and that of the lower river are effectively isolated from one another (Chalmers 1997).

Our second estuary encompasses the rivers, creeks, and marshes near Skidaway Island, GA (Figure 1). In contrast to the salt marsh estuary of the Duplin River, this area is riverine with a freshwater source at its head. Thus, the estuary is strongly influenced by saline waters at its mouth and freshwater from upstream. Such estuaries can show a well-

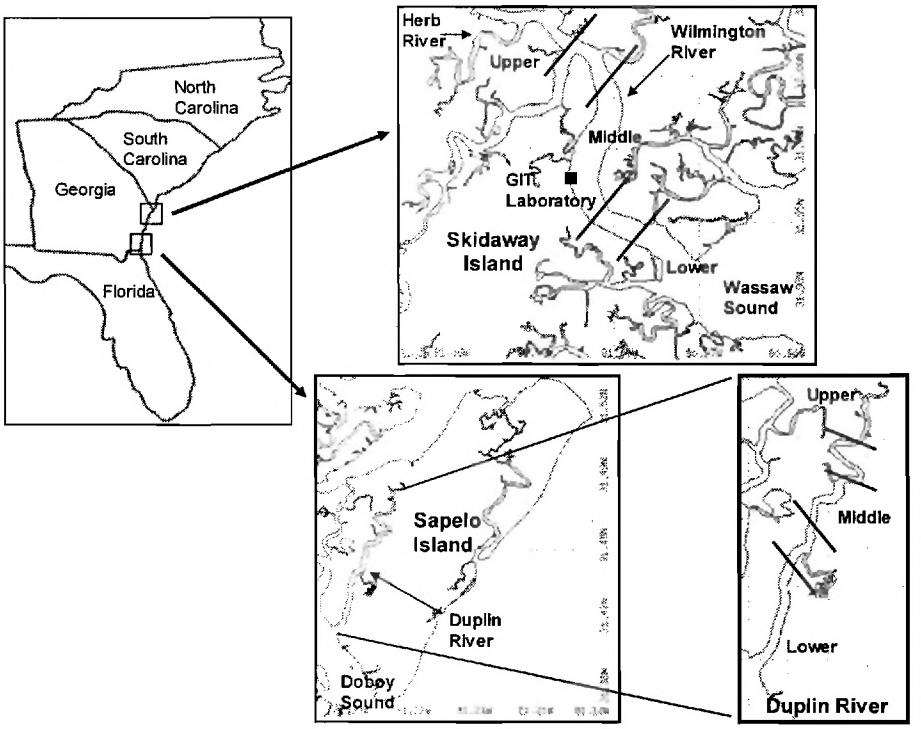

Figure 1. Map of the salt marsh estuary near Skidaway Island and Sapelo Island, GA, USA showing the Georgia Institute of Technology's marine laboratory (GIT Laboratory) and locations of the defined monitoring regions (Upper, Middle, and Lower) along the Wilmington and Duplin Rivers, respectively.

defined vertical salinity stratification where freshwater overrides more dense salt water, thus forming salt-wedges (Bahr and Lanier 1981). When tidal flow is strong enough to prevent the freshwater source from dominating circulation patterns, a partially mixed estuary with moderate salinity stratification can occur (e.g., Altamaha and Ossabaw Sounds, Georgia) (Bahr and Lanier 1981). Our defined study area included the mouth of the Wilmington River (where it meets Wassaw Sound) up to its intersection with the Skidaway River (~ 7 km) and into a large tidal creek (Herb River). There are no known tidal prisms in this region, but recent research shows that frequent gyres of circulating water occur continuously along the river's length (C. Li, *personal communication*).

Although ~ 100 km apart, both estuaries are dominated by marsh cordgrass (*Spartina alterniflora*) in the upper tidal heights, oyster reefs (*Crassostrea virginica*) and associated biota in the middle tidal regions, and muddy sediments in the lower intertidal. The estuaries are subject to semidiurnal tides with a range of 2 – 4 meters (Bahr and Lanier 1981). Thus, depending upon tidal height, portions of the middle and/or lower intertidal are exposed on most days.

Despite the hydrodynamic differences, both estuaries appear to have stronger and more frequent physical disturbances in the lower versus upper regions due to wind-wave and tidally generated currents. The middle regions are influenced by both marine and riverine processes, thus these areas are generally more turbid throughout the tides. The upper regions incorporate the tidal creeks that drain the surrounding salt marshes and are characterized as low-energy, sedimentary environments (Bahr and Lanier 1981).

Sampling

We monitored crab density, size, and sex along estuarine gradients at our two sites as a function of season and tidal height throughout the year (August 2003 – July 2004; a second year of monitoring did not get underway due to tropical storm activity and medical emergencies). Based on our best knowledge of hydrodynamics, the rivers were divided into three regions: (1) Upper, (2) Middle, and (3) Lower, with unmonitored spaces separating each region (see Figure 1). Within each region we marked 20 oyster reef monitoring sites with 1.5 meter long PVC poles and took GPS coordinates. Monitoring areas were typically separated from each other by $\geq$ 20 meters; however, in the Upper region of the Duplin River where reef sites became sparse our monitoring sites were separated by ~ 10 meters.

During spring tides in June 2003 (Wilmington River June 2 – 7, Duplin River June 9 - 12), we initiated our monitoring efforts. At each of the 20 sites within a region we placed a 22 cm x 15 cm (~ 0.03 m^2) plastic basket at a high (top edge of the reef below *S. alterniflora*) and low (just above where oysters end and mud begins) tidal height of the oyster reef along the same vertical transect and with similar basket orientation to the water-line. The baskets were anchored using rebar stakes and cable ties, thus allowing for easy removal in the future. Baskets were small enough to "nestle" into the reef, and they had large perforated sides (nine 5.4 cm long and 1.4 cm tall halfmoon-shaped holes along length and two along width) and open top that allowed for the horizontal and vertical migration of crabs. The volume of the baskets was manageable for the subsampling of crabs in a much larger volume of oyster reef. Each basket was initially

filled to its top with site-specific oyster reef substrate, including any reef-associated organisms in that volume.

For both estuaries we monitored crabs on a quarterly basis during low tides [Wilmington River: (1) August 25 - September 3, 2003 (2) December 2 - 18, 2003 (3) April 1 - 15, 2004 (4) July 7 - 12, 2004; Duplin River: (1) September 5 - 8, 2003 (2) November 20 - 22, 2003, (3) March 16 - 18, 2004, (4) June 30 - July 2, 2004]. For the initial sampling, 10 of the 20 sites in each region for each estuary were randomly chosen and sampled. During the following sampling period, the second set of 10 sites was sampled. The sites sampled were alternated like this throughout the monitoring. In cases where baskets or all material within the baskets were lost due to physical disturbances (this happened for 5 baskets out of a total of 240 for the Duplin River and 30 out of a total of 240 for the Wilmington River, particularly in the Lower region during winter storms), the closest alternate basket was sampled. All baskets that were lost or found empty were replaced and filled. The worst-case scenario was that a site was monitored every three months. The best-case scenario was every six months. In preliminary experiments, baskets placed into these habitats achieved a stable population density of *P. armatus* in about 4 weeks. The 12 - 24 weeks between monitoring considerably exceeded the time needed for crab populations to recover from the disturbance of the previous sampling.

To monitor the crab populations, baskets were removed from their anchors and the material within each basket was transferred to a closed container. If the baskets were $\leq$ 2/3 full (due to physical disturbance), the volume was noted and all material was taken.

If the baskets were 75 - 100 % full, the volume was noted and half of the material was taken. The volume was split by turning the basket over two plastic shoebox containers (width of two shoeboxes approximated the length of one monitoring basket). The contents of one randomly chosen shoebox were taken and the remaining contents were returned to the monitoring basket. All baskets were resecured in their original positions and filled to 100% with surrounding oyster substrate.

Collected material was returned to the lab, where all crabs (native and non-native) were removed, counted, sized (carapace width or CW), sexed (see Oliveira and Masunari 1995), and preserved in 70 % ethanol. If there were < 40 crabs in a sample, all were assessed. If the sample contained > 40 crabs, they were subsampled. All of the crabs from such samples were poured into a sorting tray, separated from one another, slightly suspended with a few milliliters of water, and then 30 crabs were randomly selected, sexed, and measured.

Data Analysis

We determined the estuarine-wide density patterns of native mud crabs (*Panopeus herbstii* and *Eurypanopeus depressus* combined) and non-native porcelain crabs scaled to 1 m^2 (accounting for basket contents sampled and number of baskets in 1 m^2, ~ 30.3 baskets) along the lengths of the two rivers, as a function of tidal height and season. We analyzed the effect of location on crab densities for each of the four sampling periods using two-way ANOVA (Statview, SAS). From these analyses we could determine the

overall effect of region, tidal height, and the combination of both on the distribution of *P. armatus* and total mud crab densities throughout the year.

In addition, we looked at the distribution of *P. armatus* (males and females combined) across a range of size classes (from megalopae and those ≤ 0.9 mm CW up to those 12 - 12.9 mm CW). By plotting the mean number of crabs (± S.E.) basket^{-1} in each size class for all regions, between tidal heights, and across all seasons we could visually determine any shifts in demographics due to any or all of these parameters.

With these data, we plotted the distribution of mean densities (± S.E.) of male and female *P. armatus* basket^{-1} for our defined size classes across all region - tidal height and seasonal combinations. We also calculated an overall sex ratio for each region x tidal height plot (calculations ignored megalopae that could not be reliably sexed). We did not use a mean sex ratio for our analyses because many of the baskets did not contain any crabs or contained only male crabs. Ignoring these baskets to calculate a mean sex ratio would have limited replication and statistical power. In addition, we assessed whether or not the observed frequency of males within each region and season were significantly different from an expected frequency of 50 % by analyzing the data with a χ^2 test.

We also calculated the proportion of all females large enough to be sexually mature (≥ 3 mm CW) that were ovigerous across all environmental parameters listed above. The effect of region and tidal height on the proportion of females reproducing during a particular time of year was analyzed using two-way ANOVA. An overall proportion (for

all locations and seasons) of mature females that were gravid was calculated for comparison with the overall proportion derived from Oliveira and Masunari (1995).

It is reasonable to hypothesize that an invasion of this density could negatively affect native species, particularly crabs. Therefore, we addressed this concern by correlating the estuarine-wide densities of native mud crabs (dominated by *P. herbstii* and *E. depressus*) against densities of *P. armatus* using Fisher's r to z to determine the relation of these crabs to each other among regions, between tidal heights, and across seasons.

Mesocosms: Potential Impacts

Set up

In order to perform controlled experiments that were relative to our concerns, we recreated temperate oyster reefs in mesocosm tanks that were independently treated, experimentally manipulated, and statistically analyzed. During the summer of 2003 we performed such experiments at the Georgia Institute of Technology's marine laboratory on Skidaway Island, Georgia (Figure 1), using 10 PVC tanks (1.2 x 1.2 x 0.8 m^3, ~ 1.5 m^2 bottom area) that receive raw seawater from Wassaw Sound. These tanks were continuously filled with fresh seawater via simulated wave action from a swinging bucket at the inflow pipe of each tank. Although the mesocosms were not subject to true tidal fluctuations, flow, larval inputs, ambient light of natural reefs, and large herbivores or predators (e.g., blue crabs, stone crabs, spot), the mesocosms allowed us to manipulate the densities of porcelain crabs in a controlled, "laboratory" setting and to determine the most likely impacts the crabs could be having on native species.

The mesocosm work was designed so as to gain an understanding of how the presence of *P. armatus* impacts the growth and survivorship of oysters and mussels, alters the settlement and establishment of associated reef biota (amongst the oysters and within surrounding sediments), and changes community structure in ways not predictable *a priori*. Habitat preference assays performed in the laboratory indicated that *P. armatus* adults significantly prefer clumps of live oysters over oyster shell hash (A. Hollebone *personal observation*), therefore in all mesocosm treatments, live oysters were used. In each of ten tanks we established four oyster communities in plastic tubs (23 x 30 cm^2 bottom area). All treatments were set up in each tank so as not to confound our manipulations with individual tank flow effects. Thus, each "community" had walls (47 mm tall) of chicken wire (mesh size of 0.17 x 0.17 cm^2) that extended a few centimeters above the surface of the water to separate treatments from one another. An open top allowed for periodic sampling. Each tub was placed on top of a concrete block (20 cm high) and secured with bungee cords to raise it above the anoxic zone of the tank. Within each basket we also included three randomly placed cylindrical subsamplers that allowed for the periodic (and minimally disruptive) assessment of porcelain crab densities (to ensure maintenance of treatment over time) as well as the recruitment of native species. Subsamplers were constructed out of the lower 2 cm of a 16 oz. plastic deli container (radius = 0.04 m; bottom area = 0.005 m^2) and PVC-coated chicken wire (hexagonal shape, 4 x 2.5 cm^2). The chicken wire was cut to a height of 16 cm and bent into a cylinder (0.0008 m^3) that could be inserted into the container and secured with glue.

Each community was filled with 2 liters of live oysters that had been manually defaunated in the laboratory. In each treatment we placed 10 measured (longest length using calipers) and labeled (Floy fish tags) oysters (*Crassostrea virginica*) and mussels (*Brachidontes exustus*) in order to assess any growth or survivorship effects of porcelain crabs on co-occurring filter-feeders. The four treatments in each tank were spiked with ecologically relevant densities of adult porcelain crabs ($\geq$ 6 mm CW due to mesh size). The treatments were: (1) 0 crabs m^{-2}, (2) 700 crabs m^{-2} (50 crabs $enclosure^{-1}$), (3) 1500 crabs m^{-2} (100 crabs $enclosure^{-1}$), (4) 1500 + crabs m^{-2} (100 crabs $enclosure^{-1}$ + ~ 10 more crabs $week^{-1}$). Crab collection was a limiting step, therefore, over a period of 12 days we gradually increased their numbers in each treatment. The crabs were added proportionally according to final density and evenly distributed among the treatments.

Each treatment was randomly assigned an initial position in each tank, but the array was rotated counterclockwise in space every 2-3 days to avoid spatial bias. We simulated tidal exposure by draining the tanks for 2.5 - 4 hours a day during the late morning (to avoid afternoon heat). Small holes (6-9) drilled along on bottom surface of each tub allowed for the slow drainage of water. Preliminary experimental efforts in these tanks indicated that long exposure time and/or exposure during the extreme heat of the day killed many of the crabs.

Sampling

Throughout the 12 week duration of the experiment, we periodically sampled the established communities in order to ensure that the porcelain crab densities remained at

intended levels. At 2, 3, 4, 6, 7, 9 and 12 weeks we randomly selected one of the subsamplers from each basket, counted the number of adult porcelain crabs within each cylinder and replaced the sampler and contents to the original position. Each sampling period we looked at a different sampler, rotating through the series twice. The density of crabs sampled was scaled up to the size of the basket (10.8 samplers basket^{-1}) and porcelain crabs were added to particular treatments if the numbers were low based on the mean density for that treatment across all tanks.

In addition to assessing porcelain crab densities at the above times, we also identified the native species that were in each of the chosen samplers. These data allowed us to assess the effect of the crabs on the recruitment and success of native species (richness). These data were analyzed using a repeated-measures ANOVA.

We also assessed the effect of the crab on the growth and survivorship of the live oysters and mussels that were originally measured and labeled. At 4 weeks, we measured the longest length and noted the condition of all labeled mussels and oysters that could be acquired without disturbing the other contents of the baskets. Upon termination of the experiment (12 weeks), we retrieved and measured all remaining labeled oysters and mussels (many tags were lost over time). We statistically assessed whether or not the crabs had any affect on oyster and mussel growth and survivorship by pooling data for the 1500 and 1500 + crabs m^{-2} treatments and ignoring the 700 crabs m^{-2} treatment (see Results) and using unpaired *t* tests to compare differences between extremes.

In addition to assessing the impact of porcelain crab presence on the growth and survivorship of co-occurring filter feeders, we also assessed the crab's impact on what grows on oyster surfaces. *P. armatus* lives in very close association with the surfaces of oysters. They have been observed to actively scrape the surfaces of oysters with their large chelipeds and feed on the particles scraped off as well as to clip and feed upon small algal spores (A. Hollebone *personal observation*, Kropp 1981). Therefore, at 0, 2, 4, 6, 8, and 12 weeks we took samples of the microalgal growth on the oyster surfaces for chlorophyll *a* analysis. We used chlorophyll *a* data as a proxy for microalgal biomass (see also Duffy *et al.* 2001). Using 1 cm gradations of the inner length and width of the baskets, we selected three random points of oyster surface within in each basket. During a period of simulated low tide we scraped (using a metal spatula) the material from these 1 cm^2 areas, combined the scrapings from one basket into a 1.5 mL tube, and removed samples from the light so as to avoid any further growth. The samples were returned to the lab and stored at 4° C for no more than 24 hours until filtration could be performed. Material from each basket was individually filtered onto glass microfiber GF/F filters. The filters were then folded into aluminum foil and stored at -20° C for $\leq$ 30 days.

For the actual extraction of the photosynthetic pigments, we followed the acetone extraction of Parsons *et al.* (1984). Due to the relatively large quantities of material we obtained, we extracted our samples for 24 hours at -20° C prior to fluorometric analysis. After this extraction period, the solvent and filter for each sample were sonicated for 30-60 seconds and allowed to extract on ice for 20-30 minutes more. Tubes were spun down in a centrifuge for 7 minutes. The high concentrations of pigments in our samples were

diluted with 90% acetone (HPLC grade) so as not to overwhelm the fluorometer readings (Turner Designs 10-AU Fluorometer). Periphyton (chlorophyll *a*) and phaeophyton (following the addition of 1 drop of 2M HCl to sample) content were calculated by an adaptation of Parsons *et al.* (1984). Chlorophyll *a* measurements were compared among treatments over time using a repeated-measures ANOVA.

At 8 weeks we began to notice an increase in the growth of green alga on oyster surfaces. These macrophytes including *Ulva spp.* and *Enteromorpha spp.*, predominantly grew where there were porcelain crabs. Algal percent cover was determined by using a quadrat with 100 randomly selected points. We also assessed algal cover at 12 weeks in the same manner. These data were analyzed using unpaired *t* tests comparing 0 crabs m^{-2} versus 1500 crabs m^{-2} (pooled data for 1500 and 1500+ crabs m^{-2} treatments).

RESULTS

Monitoring

Overall Densities

In the Duplin River estuary, *P. armatus* densities ranged from ~ 15 – 3,900 m^{-2} in warmer months (Figure 2). During the colder months (reflected in March 2004 data) densities decreased ~ 64 - 69% in the Upper region, ~ 85 - 93% in the Middle region, and ~ 91 - 95% in the Lower region from November 2003 levels and ranged between ~ 45 – 450 crabs m^{-2}. In September, November, and July there was a significant region effect (two-way ANOVA, $p \leq 0.003$), densities increased from Upper regions to the Middle and Lower regions, but no significant effect was detected in March ($p = 0.195$) when densities

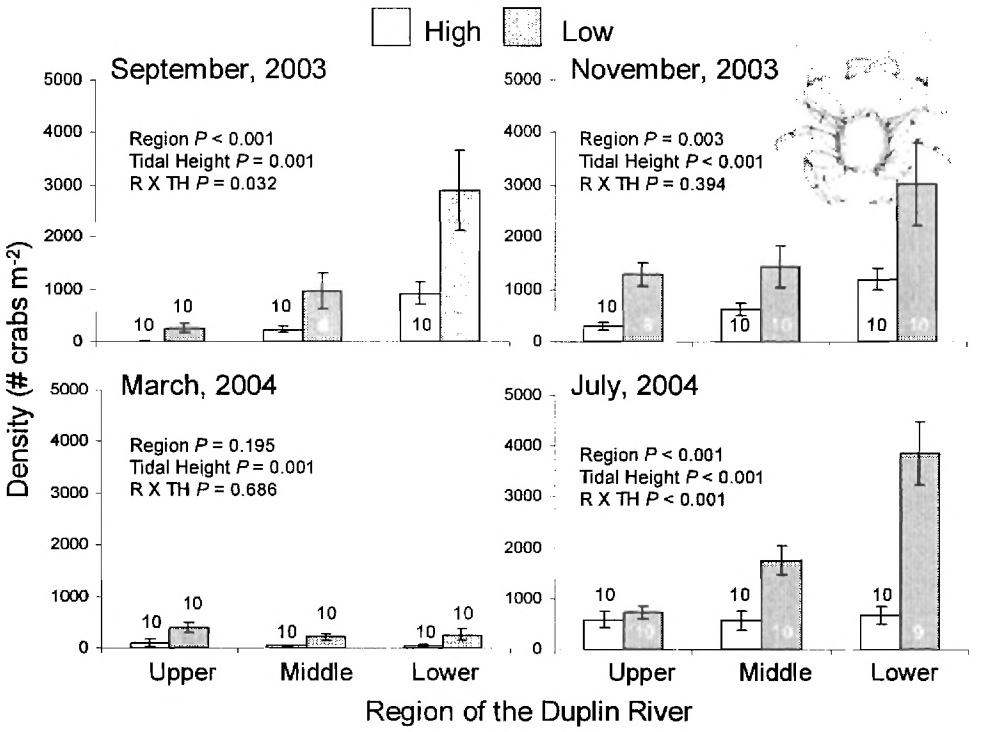

Figure 2. Densities of *Petrolisthes armatus* in the Duplin River among estuarine regions (Upper, Middle, Lower), between tidal heights (white = high intertidal; grey = low intertidal), and throughout the year scaled to 1 m^2. Numbers within or directly above bars indicate the number of replicate baskets sampled. Error bars indicate ± 1 S.E. P values derived from a two-way ANOVA.

were at their lowest. Tidal height had a significant effect on porcelain crab densities across all seasons (two-way ANOVA, $p \leq 0.001$ for all 4 seasons) with crabs being most dense in the low intertidal. We found a significant region x tidal height interaction in September ($p = 0.032$) and July ($p < 0.001$). In both cases, density differences between low (dense) and high (sparse) tidal areas grew greater as we moved down the estuary toward the sound.

In the Wilmington River estuary, densities of *P. armatus* in the Wilmington River estuary were higher during warmer months, with mean densities ranging between ~ 1,000 - 12,000 crabs m^{-2} (Figure 3). During colder months (reflected in the April 2004 data), densities decreased ~ 95 - 97% in Upper region, ~ 97 - 99.7% in Middle region, and ~ 89 - 98% in Lower region from December 2003 levels and averaged only ~ 20 – 170 crabs m^{-2}. A region effect was significant only during June (two-way ANOVA, $p < 0.001$), but there were strong trends in August and December ($p = 0.083$ and $p = 0.056$, respectively). This significant region effect in June was due to greatest densities occurring in the Middle region. Tidal height significantly affected *P. armatus* densities in April ($p = 0.006$) and June ($p = 0.045$), but not during the other monitoring periods. In the Wilmington River estuary there was never a significant region x tidal height interaction on porcelain crab densities.

Demographics

By plotting the distribution of crabs among size classes for all region and tidal height x season combinations, we obtained an overall view of the crab's demographic

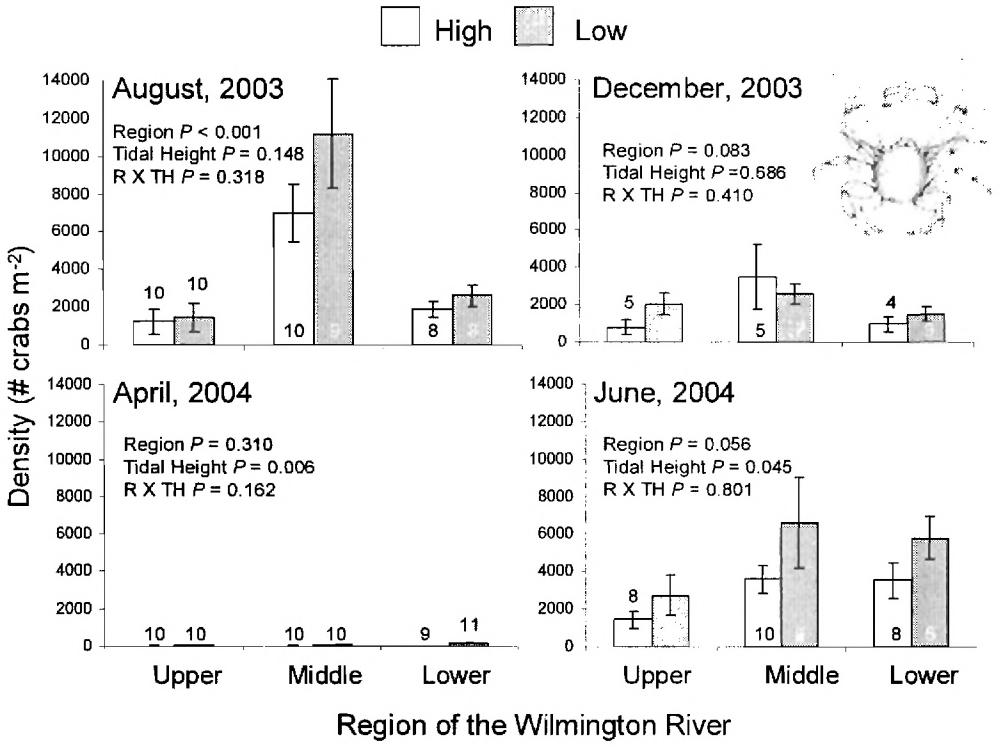

Figure 3. Densities of *Petrolisthes armatus* in the Wilmington River among estuarine regions (Upper, Middle, Lower), between tidal heights (white = high intertidal; grey = low intertidal), and throughout the year scaled to 1 m^2. Numbers within or directly above bars indicate the number of replicate baskets sampled. Error bars indicate ± 1 S.E. P values derived from a two-way ANOVA.

fluctuations. In both estuaries, recruitment (appearance of crabs < 3 mm CW) occurred primarily during warmer months (Figures 4 and 5). Recruits (megalopae), juveniles, and gravid females (Figures 6 and 7) were essentially absent in March and April, following the coldest months of the year. Some crabs of reproductive size (≥ 3 mm CW) persisted through the winter. Although crabs were generally more abundant in the low versus high intertidal, size class distributions were visually similar for the two tidal habitats.

In addition to determining the densities of male versus female crabs for each region x tidal height combination (in A. Hollebone's *PhD. Dissertation*), we also looked at the ratio of males to females (Table 1). For both estuaries, χ^2 analyses across all spatial and temporal combinations did not indicate that any of the observed sex ratios were significantly different from the expected ratio of 1 : 1 (Duplin River, $p = 0.317 - 0.970$ and Wilmington River, $p = 0.230 - 0.988$ for all combinations). There was an equal number of males and females over all region x tidal height combinations and seasons ~ 29 % of the time in the Duplin River and ~ 21 % of the time in the Wilmington River.

Reproduction

Overall, we found that a large proportion of mature females were gravid in the warm months of the year (Duplin River: ~ 40 – 80 % in September and ~ 20 – 65 % in July, Wilmington River: ~ 70 – 90 % in August and ~ 25 – 75 % in June). Females were rarely gravid during the colder months (Duplin River: ~ 0 – 10 % in November and March, Wilmington River: ~ 0 – 15 % in December and April) (Figures 6 and 7). The overall

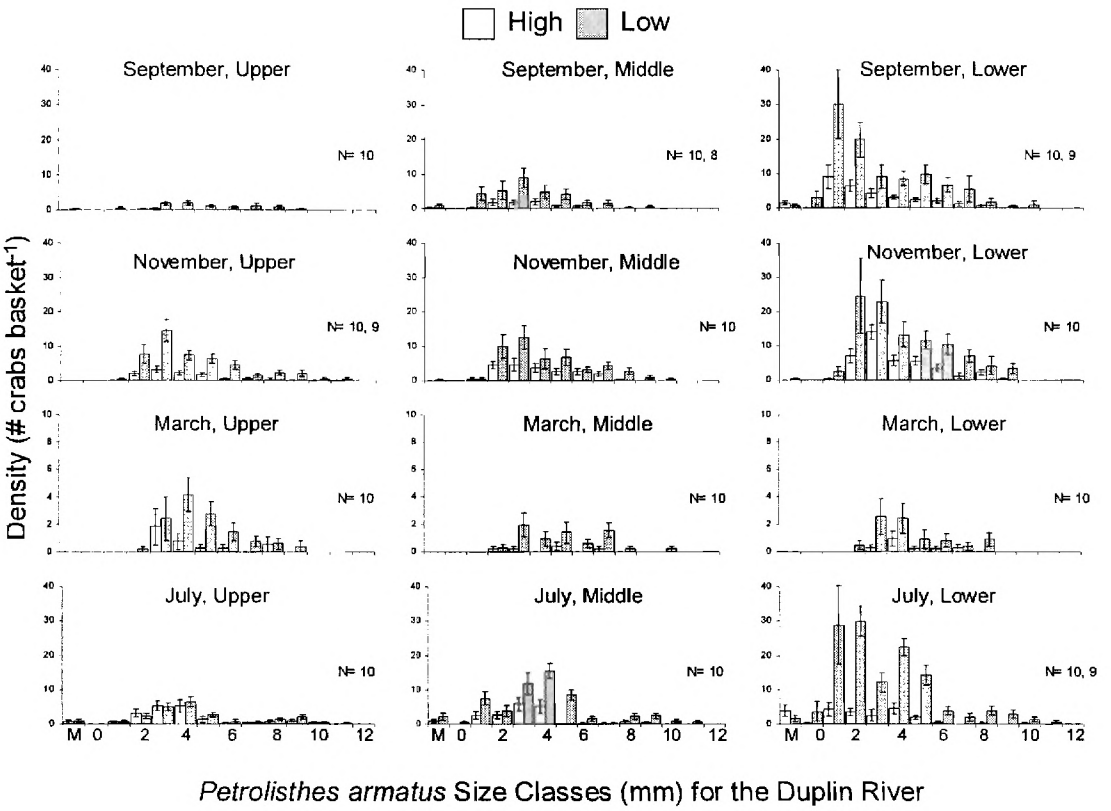

Figure 4. Size classes of *Petrolisthes armatus* in the Duplin River among estuarine regions (Upper, Middle, Lower), between tidal heights (white = high intertidal; grey = low intertidal, and throughout the year scaled to 1 basket. Units for size classes are in millimeters carapace width. Megalopae are newly settled crabs that cannot be sexed with certainty.

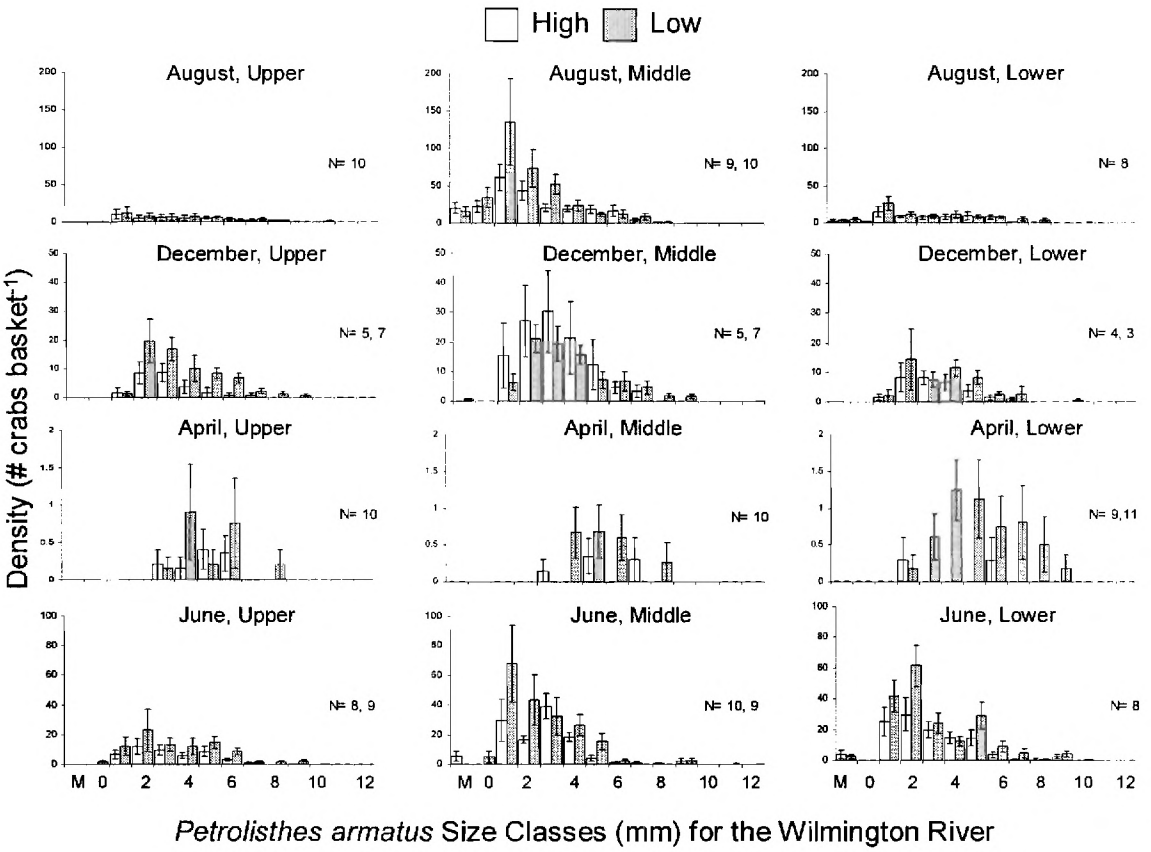

Figure 5. Size classes of *Petrolisthes armatus* in the Wilmington River among estuarine regions (Upper, Middle, Lower), between tidal heights (white = high intertidal; grey = low intertidal, and throughout the year scaled to 1 basket. Units for size classes are in millimeters carapace width. Megalopae are newly settled crabs that cannot be sexed with certainty.

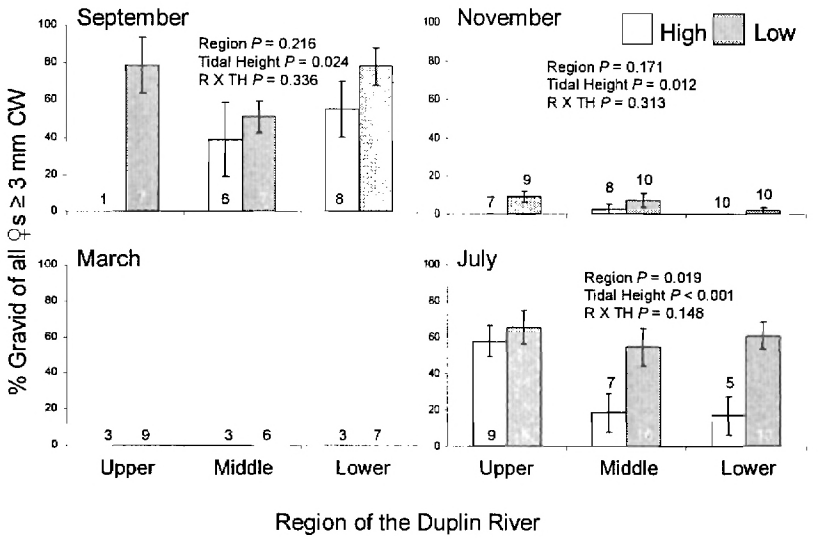

Figure 6. Proportion of all potentially mature females (≥ 3 mm CW) that are gravid in the Duplin River among estuarine regions (Upper, Middle, Lower), between tidal heights (white = high intertidal; grey = low intertidal), and throughout the year. Numbers within or directly above bars indicate replicate number. Error bars indicate ± 1 S.E. P values derived from a two-way ANOVA.

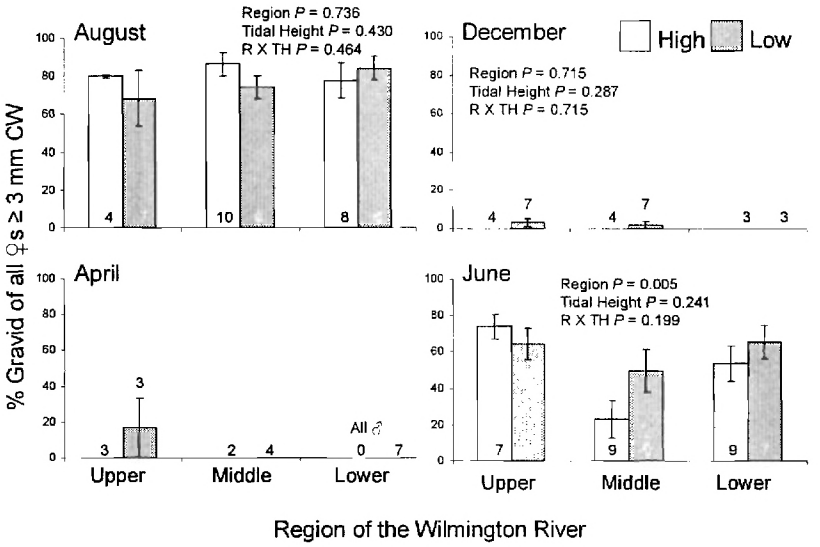

Figure 7. Proportion of all potentially mature females (≥ 3 mm CW) that are gravid in the Wilmington River among estuarine regions (Upper, Middle, Lower), between tidal heights (white = high intertidal; grey = low intertidal), and throughout the year. Numbers within or directly above bars indicate replicate number. Error bars indicate ± 1 S.E. P values derived from a two-way ANOVA.

Table 1

Overall sex ratios (male:female) of *Petrolisthes armatus* (>1mm CW) and chi squared P values

Sampling Period	Duplin River			Wilmington River		
August - September 2003	**Male:Female**	**Ratio**	**χ^2 P value**	**Male:Female**	**Ratio**	**χ^2 P value**
Upper High	1:1	1.00	0.317	53:51	1.04	0.780
Upper Low	20:20	1.00	0.944	60:66	0.91	0.677
Middle High	25:11	2.27	0.849	122:132	0.92	0.447
Middle Low	53:46	1.15	0.970	114:133	0.86	0.558
Lower High	76:68	1.12	0.809	88:112	0.79	0.230
Lower Low	113:110	1.03	0.892	115:112	1.03	0.833
November - December 2003						
Upper High	20:24	0.83	0.929	24:14	1.71	0.427
Upper Low	98:93	1.05	0.404	83:61	1.36	0.633
Middle High	49:37	1.32	0.672	60:39	1.54	0.320
Middle High	82:93	0.88	0.947	97:104	0.93	0.988
Lower High	98:78	1.26	0.834	37:22	1.68	0.576
Lower Low	106:109	0.97	0.692	28:32	0.88	0.511
March - April 2004						
Upper High	9:5	1.80	0.644	3:3	1.00	0.717
Upper Low	30:21	1.43	0.750	3:7	0.43	0.497
Middle High	4:3	1.33	0.919	1:3	0.33	0.368
Middle Low	17:14	1.21	0.911	6:4	1.50	0.751
Lower High	2:6	0.33	0.446	2:0	100% male	0.317
Lower Low	21:19	1.11	0.563	14:15	0.93	0.961
June - July 2004						
Upper High	33:45	0.73	0.965	73:64	1.14	0.720
Upper Low	49:51	0.96	0.651	72:87	0.83	0.407
Middle High	49:36	1.36	0.721	142:139	1.02	0.602
Middle Low	109:116	0.94	0.493	112:132	0.85	0.646
Lower High	34:30	1.13	0.636	138:111	1.24	0.771
Lower Low	135:186	0.73	0.792	100:99	1.01	0.688

proportion of mature females that were gravid across all locations and seasons was 30.5% in the Duplin River and 48.1% in the Wilmington River.

In the Duplin River tidal height significantly affected the distribution of gravid females for all three seasons in which we found such females (two - way ANOVA, $p \leq 0.024$ for September, November, and July); gravid females were more abundant in the low versus high intertidal. In contrast, tidal height never significantly affected gravid females in the Wilmington River. Region significantly affected reproduction in both estuaries during the June – July period (two - way ANOVA, $p = 0.019$ for Duplin River and $p = 0.005$ for Wilmington River) with the proportion of ovigerous females being high in the Upper regions. There was never a significant region x tidal height interaction in either estuary.

Biological Factors

The two most common mud crabs within the oyster reefs we monitored, *P. herbstii* and *E. depressus*, were abundant throughout the year (~ 600 – 1,820 crabs m^{-2} in the Duplin River and ~ 360 – 1,520 crabs m^{-2} in the Wilmington River). Their distribution and seasonality differed in some aspects from the patterns for *P. armatus* (Figures 8 and 9). Mud crabs were less seasonal than *P. armatus*. While *P. armatus* were 10 – 100 X more common in warmer months than in the early spring, mud crabs were only 1 – 3 X more common in warm periods. For the Duplin River there was a tendency for higher mud crab densities in the Middle and Lower versus Upper regions, but this was significant only in July (two – way ANOVA, $p = 0.003$). A significant tidal height effect occurred only in November ($p = 0.048$) when there were slightly more mud crabs in the high

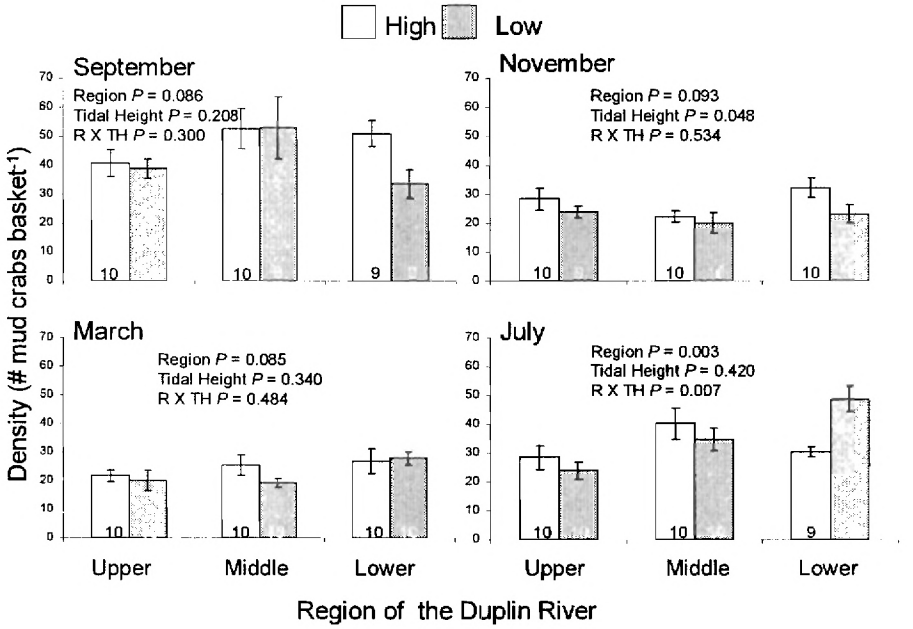

Figure 8. Overall densities of native mud crabs (*Panopeus herbstii* and *Eurypanopeus depressus*) in the Duplin River among estuarine regions (Upper, Middle, Lower), between tidal heights (white = high intertidal; grey = low intertidal), and throughout the year scaled to 1 m^2. Numbers within or directly above bars indicate replicate number. Error bars indicate ± 1 S.E. P values derived from a two-way ANOVA.

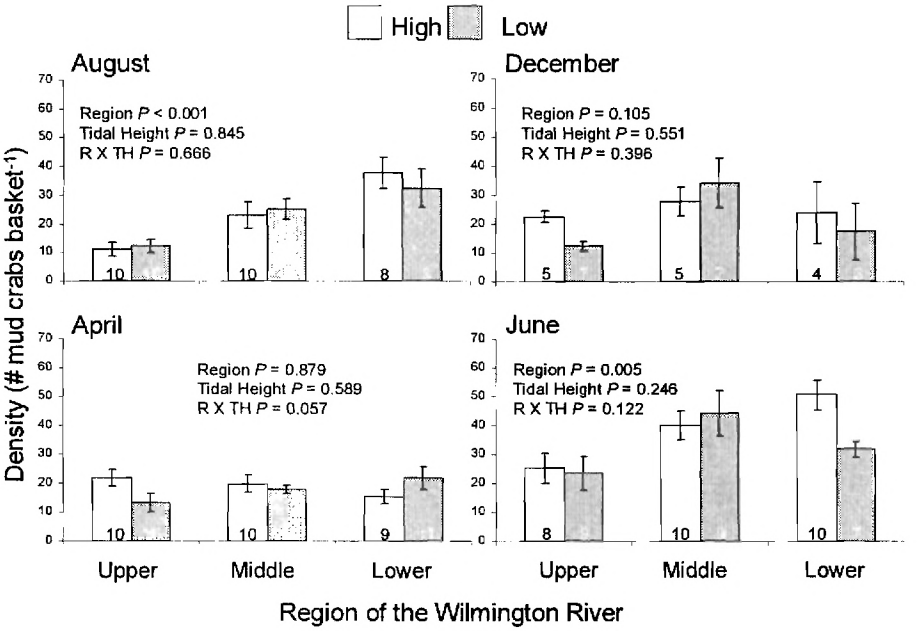

Figure 9. Overall densities of native mud crabs (*Panopeus herbstii* and *Eurypanopeus depressus*) in the Wilmington River among estuarine regions (Upper, Middle, Lower), between tidal heights (white = high intertidal; grey = low intertidal), and throughout the year scaled to 1 m^2. Numbers within or directly above bars indicate replicate number. Error bars indicate ± 1 S.E. P values derived from a two-way ANOVA.

versus the low intertidal. There was a significant region x tidal height effect in July (p = 0.007) with the Middle and Upper regions having more mud crabs in the high intertidal and the Lower region having more crabs in the low intertidal. For the Wilmington River we saw a similar regional effect on mud crab densities (two – way ANOVA, August and June $p \leq 0.005$ and December $p = 0.105$) where there were slightly more mud crabs in the Middle and Lower regions than in the Upper region with the exception of April (p = 0.879). There was no significant effect of tidal height or region x tidal height on mud crab densities and distribution in the Wilmington River.

Correlations of mud crab densities basket^{-1} with *P. armatus* densities basket^{-1} across each region, tidal height, and season, indicated several significant positive relationships but no significant negative relationships (Table 2 a, b). Of the 24 contrasts in each estuary system, five (Wilmington River) or six (Duplin River) were significantly positive, with all but one occurring in the warmer periods of the year (June - July and August - September). No significant negative correlations occurred in any location or season.

Mesocosms

Maintenance of Treatments

Our ability to maintain porcelain crab density treatments in the mesocosm tanks due to unknown factors was not possible after 8 weeks (Figure 10). Therefore, most of our analyses of the impacts of porcelain crabs on oyster reefs and related biota were analyzed up to 8-9 weeks. Some parameters, though, could only be assessed at 12 weeks when the experiment was terminated (showed lag time). We were never able to maintain crabs

Table 2a: Correlations (Fisher's r to z) of mud crabs vs. *P. armatus* for the Duplin River estuary. Boxed P values indicate significant correlations.

	N	Correlation	Z	P value
Duplin River: September 2003				
Upper High	10	0.079	0.210	0.834
Upper Low	10	0.269	0.731	0.465
Middle High	10	0.601	1.838	0.066
Middle Low	8	0.780	2.339	**0.019**
Lower High	9	0.837	2.966	**0.003**
Lower Low	9	0.294	0.742	0.458
Duplin River: November 2003				
Upper High	10	0.208	0.557	0.577
Upper Low	9	-0.473	-1.260	0.208
Middle High	10	0.116	0.309	0.758
Middle Low	10	0.366	1.015	0.310
Lower High	10	-0.247	-0.667	0.505
Lower Low	10	0.760	2.636	**0.008**
Duplin River: March 2004				
Upper High	10	-0.464	-1.330	0.184
Upper Low	10	0.306	0.836	0.403
Middle High	10	-0.574	-1.728	0.084
Middle Low	10	-0.136	-0.363	0.716
Lower High	10	-0.353	-0.975	0.329
Lower Low	10	0.057	0.150	0.880
Duplin River: July 2004				
Upper High	10	0.736	2.492	**0.013**
Upper Low	10	0.212	0.569	0.569
Middle High	10	0.919	4.187	**<0.001**
Middle Low	10	0.157	0.419	0.675
Lower High	9	0.470	1.251	0.211
Lower Low	10	0.826	3.111	**0.002**

Table 2b: Correlations (Fisher's r to z) of mud crabs vs. *P. armatus* for the Wilmington River estuary. Boxed P values indicate significant correlations.

		N	Correlation	Z	P value
Wilmington River: August 2003					
	Upper High	10	0.780	2.769	**0.006**
	Upper Low	10	0.791	2.842	**0.005**
	Middle High	10	-0.100	-0.267	0.790
	Middle Low	9	0.164	0.406	0.685
	Lower High	8	0.333	0.774	0.439
	Lower Low	8	0.712	1.992	**0.046**
Wilmington River: December 2003					
	Upper High	5	0.384	0.572	0.568
	Upper Low	7	0.276	0.567	0.571
	Middle High	5	0.433	0.655	0.513
	Middle Low	7	0.157	0.316	0.752
	Lower High	4	0.638	0.754	0.451
	Lower Low	3	0.494	0.000	>0.999
Wilmington River: April 2004					
	Upper High	10	-0.423	-1.193	0.233
	Upper Low	10	0.273	0.742	0.458
	Middle High	10	-0.095	-0.253	0.800
	Middle Low	10	0.467	1.340	0.180
	Lower High	9	0.342	0.872	0.383
	Lower Low	11	-0.058	-0.164	0.870
Wilmington River: June 2004					
	Upper High	8	0.717	2.016	**0.044**
	Upper Low	9	0.355	0.910	0.363
	Middle High	10	0.244	0.659	0.510
	Middle Low	9	0.741	2.335	**0.020**
	Lower High	10	0.302	0.826	0.409
	Lower Low	7	-0.109	-0.219	0.827

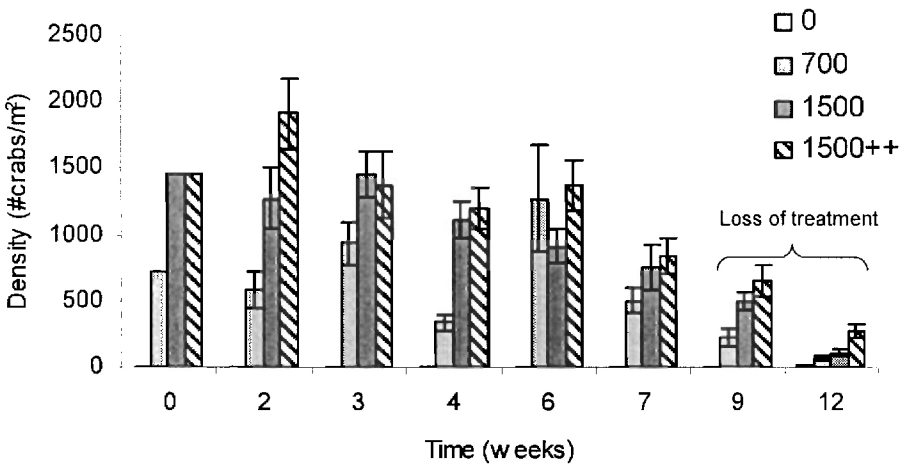

Figure 10. Mean densities of adult *Petrolisthes armatus* sampled from the four mesocosm treatments over time. All data scaled to 1 m^2. Numbers in the key indicate crabs m^{-2}. N = 10 for all treatments and times. Bars indicate ± 1 S.E. By 9 weeks, treatments could no longer be maintained.

above 1500 crabs m^{-2} and the densities for the 700 crabs m^{-2} treatments fluctuated greatly and were not reliable. Therefore, we pooled all data for the 1500 and 1500+ crabs m^{-2} treatments and only compared 0 versus 1500 crabs m^{-2} treatments.

Native Species Richness

We assessed the effect of porcelain crab presence on the recruitment of native taxa and found that richness increased over time across all treatments with a final number of ~ 7 species $basket^{-1}$ (e.g., *Boonea spp.*, *P. herbstii*, *E. depressus*, *Sesarma cinereum*, *Cliona spp.*, *Brachidontes exustus*, amphipods, tannaids, polychaetes) (Figure 11). Many of the organisms typically found in the field were not represented likely because their larva were not pulled into the water system or their larva settled out in the pipes that supplied water to the tanks. There was no significant effect of crab presence on species richness (repeated measures ANOVA, $p = 0.0607$), but there were significant time ($p < 0.0001$) and treatment x time ($p = 0.0002$) effects. If we were to only assess what happened over time, we would likely conclude that there was no impact of *P. armatus* on native species. Instead, we went a step further and looked more closely at species-specific effects.

Oyster and Mussel Growth and Survivorship

We determined that the presence of porcelain crabs can potentially have significant negative effects on oyster growth, particularly for oysters in smaller size classes (Figure 12). At 4 weeks we found that there was a significant difference in growth of oysters that were initially $\leq$ 60mm in length (unpaired *t* test, $p = 0.040$) in communities with and without *P. armatus*. Those oysters in the presence of the porcelain crabs grew less than

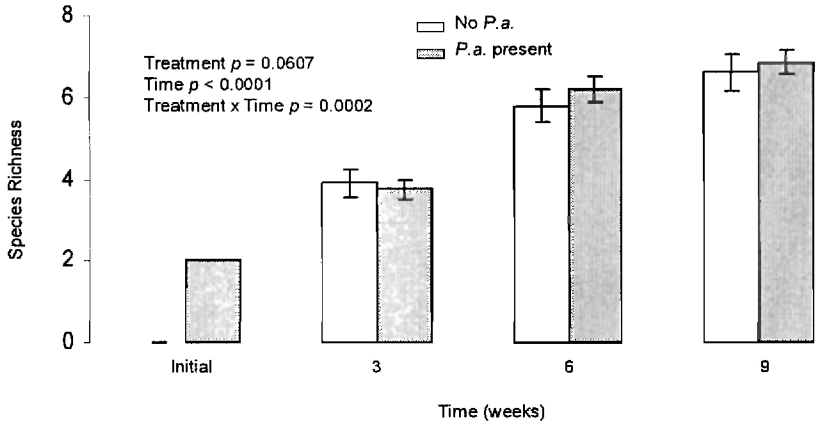

Figure 11. Native species richness in mesocosms over time. Comparisons between 0 (No *P.a.*) and 1500 (*P.a.* present) crabs m^{-2}. Error bars indicate ± 1 S.E. P values derived from a repeated measures ANOVA (N = 10, 20).

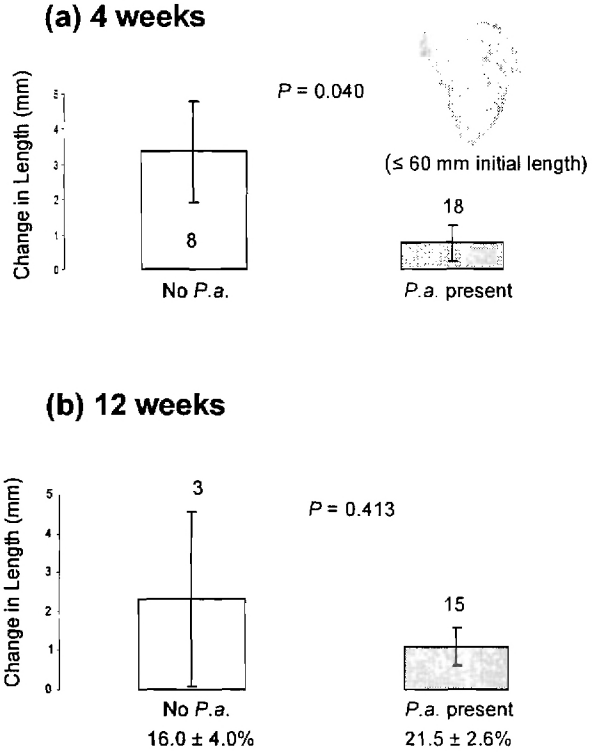

Figure 12. Growth of oysters with initial length ≤ 60 mm in the mesocosm experiment. Data from 4 weeks (a) and 12 weeks (b). Comparisons between 0 (No *P.a.*) and 1500 (*P.a.* present) crabs m^{-2}. Numbers within or directly above bars indicate replicate number. Error bars indicate ± 1 S.E. P values derived from t tests. Percent values at the bottom of the figure indicate mean proportion ± S.E. of live and labeled oysters that were retrieved at the end of the experiment.

oysters in areas without crabs. Analysis of oysters across an entire range of initial lengths (~ 30 - 90 mm length) did not show this trend. At 12 weeks we were only able to recover 16 - 21.5% of the originally labeled oysters and, thus, lost replication power when following the smaller size classes. Overall, oysters ≤ 60 mm in initial length grew ~ 1 mm where there were porcelain crabs and ~ 2.5 mm where there were no porcelain crabs.

Mussels did not follow the same pattern as oysters. Mussels grew ~ 1.5 mm in length by 4 weeks and ~ 4 mm in length by 12 weeks. There did not appear to be any impact of porcelain crab presence on growth or survivorship of mussels. Approximately 69 - 74% of mussels survived in both crab treatments over 12 weeks (Figure 13).

Microalgal Biomass

We determined that the presence of *P. armatus* potentially moderates the growth and persistence of microalga on oyster shells. Initial chlorophyll *a* samples indicated that in all treatments oysters were covered with ~ 4 μg cm^{-2} of algal material, but seasonal fluctuations led to increases in these concentrations where there were no crabs within 2 weeks of experimentation (Figure 14). In areas with porcelain crabs microalgal biomass consistently remained within the 6 - 8 μg cm^{-2} range. By 4 weeks the peak in algal growth for communities without crabs began a steady decline that lasted through the remainder of the study. At 8 weeks, chl *a* measurements fell below those in porcelain crab treatments. Overall, there was no significant treatment effect on algal growth (repeated measures ANOVA, $p = 0.0884$), but there were significant time and treatment x time effects ($p < 0.0001$).

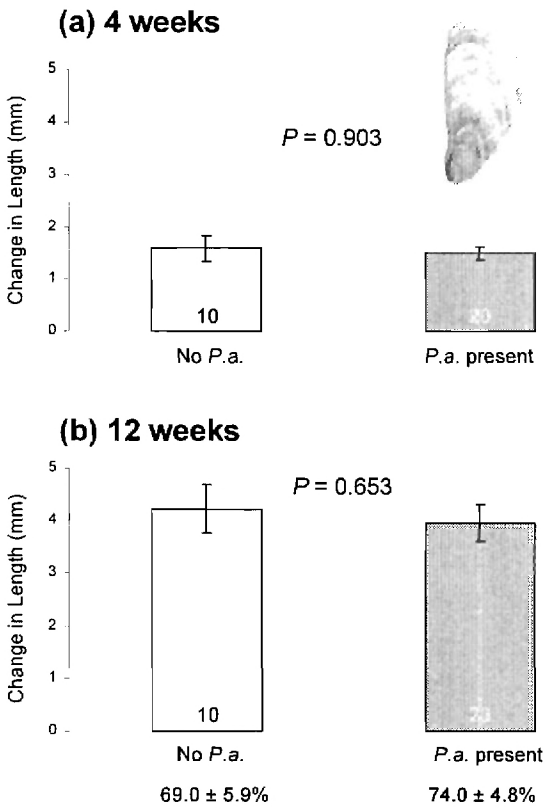

Figure 13. Growth of mussels in the mesocosm experiment. Data from 4 weeks (a) and 12 weeks (b). Comparisons between 0 (No *P.a.*) and 1500 (*P.a.* present) crabs m^{-2}. Numbers within bars indicate replicate number. Error bars indicate ± 1 S.E. P values derived from t tests. Percent values at the bottom of the figure indicate mean proportion ± S.E. of live and labeled oysters that were retrieved at the end of the experiment.

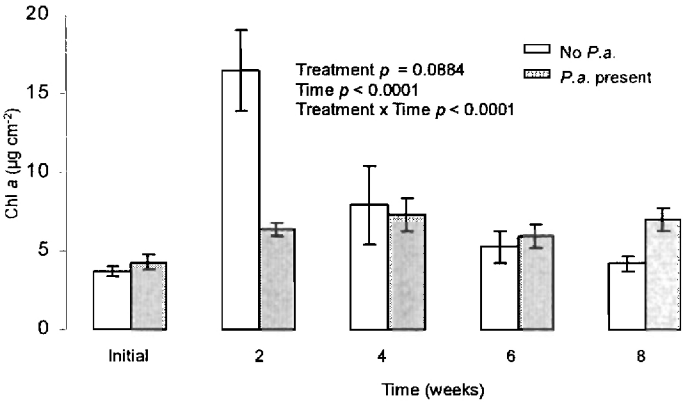

Figure 14. Changes in microalgal biomass (Chl *a*) on the surfaces of oysters over time. Comparisons between 0 (No *P.a.*) and 1500 (*P.a.* present) crabs m^{-2}. Error bars indicate ± 1 S.E. P values derived from a repeated measures ANOVA (N = 10, 20).

Macroalgal Growth

In addition to the growth of microscopic alga and diatoms on oyster surfaces, we also observed the growth of large macrophytes on oysters, particularly in the presence of *P. armatus*. At 8 weeks we noticed the appearance of large leaves of *Ulva spp.* and *Enteromorpha spp.* within our experimental units. When we assessed macroalgal percent cover we found ~ 20 % cover in areas devoid of porcelain crabs and ~ 60 - 65% in areas with porcelain crabs (Figure 15). These data were significantly different from one another (unpaired t test, $p = 0.0001$). This trend held until week 12 when the experiment was terminated. By this time percent cover dropped but was still significantly different between the two treatments (~ 5% versus ~ 18 - 25%, unpaired t test, $p = 0.0002$).

DISCUSSION

Invasive species pose serious threats to the function and maintenance of native communities (Carlton and Geller 1993). In recent years there have been more frequent invasions than at any previous time in the natural progression of species' ranges due to the circumvention or breakdown of physical and biological barriers by human activities. Nonindigenous species have fundamentally changed the community composition of areas such as the San Francisco and Chesapeake Bays and continue to threaten coastal systems (Ruiz *et al.* 1999). Successful invasions in marine habitats have been studied far less than those in terrestrial environments but are of such a magnitude that they may be leading to profound ecological changes in the ocean (Grosholz 2002).

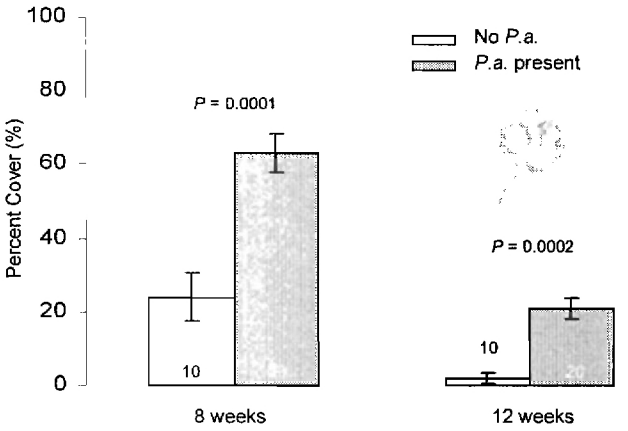

Figure 15. Macroalgal abundance in percent cover at 8 and 12 weeks. Comparisons between 0 (No *P.a.*) and 1500 (*P.a.* present) crabs m^{-2}. Numbers within or directly above bars indicate replicate number. Error bars indicate ± 1 S.E. P values derived from t tests.

Marine invaders are not limited to specific taxa or functional groups. Crabs, in particular, have had wide-ranging impacts on native species' traits, population levels, and community composition and function. Introduced crabs have affected the induction of morphological defenses (Trussell and Nicklin 2002), trait-mediated indirect effects on prey (Trussell *et al.* 2002), the evolution of defenses (Vermeij *et al.* 1981), and even the success of bivalve fisheries (Walton *et al.* 2002). Our data suggest that the green porcelain crab has invaded oyster reefs of the SAB at densities up to 40 X greater than the highest densities documented in their native habitat (Oliveira and Masunari 1995). They are persistent throughout the year, rapidly colonizing, growing, reproducing, and settling during warm periods. Currently, *Petrolisthes armatus* is the single most abundant crab on the dominant hard substrate habitat of shallow waters in the South Atlantic Bight. Its densities can exceed by 7 X the highest combined densities of the two most common native mud crabs, *P. herbstii* and *E. depressus*. Their potential impacts are wide-ranging from the depression of oyster growth to the facilitation of macroalgal growth in both mesocosm and field (A. Hollebone *PhD. dissertation*) experiments. At this time, though, such effects are not apparent in naturally occurring oyster reefs.

Population Dynamics

Overall Densities

In about a decade, *P. armatus* has increased from mere individuals m^{-2} to mean densities of several thousand individuals m^{-2} in warmer portions of the year (with patches of up to ~ 32,000 m^{-2}). Their densities generally increased from Upper to Middle and Lower regions of the estuaries, and there were more crabs in the low versus the high intertidal

during warmer months. During colder months crab densities declined by 64 - 95 % in the Duplin River and by 89 – 99.7 % in the Wilmington River across all region x tidal height combinations. Size class distributions indicated that a few adult porcelain crabs persisted through the winter for all regions and in both estuaries. These numbers included males and females of reproductive size, leaving a viable local population year-round.

Tidal Height Distribution

Spatial and temporal patterns of density and reproduction could be affected by physiological or biological constraints of the crabs. Previous work with *Petrolisthes cinctipes* and *P. eriomerus* in Washington, USA and British Columbia, Canada showed that both biotic (competition and predation) and abiotic (thermal tolerance and substrate composition) factors affected distribution in the intertidal (Jensen and Armstrong 1991). Although both species appeared to be equally susceptible to dessication, *P. eriomerus* was particularly susceptible to thermal stress during low tides and aerial exposure. The consistently higher densities of *P. armatus* in the low intertidal at the sites we monitored may be explained by longer exposure times and increased temperatures in the high intertidal. In addition, extreme low tides are not a sudden event, therefore individuals are subject to increasing thermal stress for a number of days prior to the lowest low tide. As the tides progress over time, the crabs would likely move down the tidal gradient and into the lower intertidal (Jensen and Armstrong 1991).

Regional Distribution

The regional patterns we observed for *P. armatus* may be explained by the thermal environment of the entire estuary which is strongly influenced by hydrography (Bahr and Lanier 1981). Typically, in the Lower regions of the estuary temperature does not show a pronounced vertical gradient but is subject to daily fluctuations. During the warmer months of the year, the cooler ocean water temperatures have a moderating effect on the Lower estuary, whereas water temperatures of the marsh creeks and Upper estuary are higher due to the heating of dark sediments during low tides (Bahr and Lanier 1981). Data collected in 2003 (during part of the monitoring program) from the SINERR hydrographic monitoring stations (http://gce-lter.marsci.uga.edu/portal /sinerr_hydro) at Sapelo Island, Georgia support Bahr and Lanier's generalizations (1981). From June to September, water of the Upper (~ 25 – 32.5 °C) Duplin River estuary was generally hotter than the Lower region (~ 25 – 30 °C). By November and December water of the Upper region (~ 8 – 25 °C) was similar to, or colder than, the Lower region (~ 10 – 25 °C).

In its native range, *P. armatus* thrives under thermal conditions within the range of Spring – Autumn temperatures in the SAB (16 – 29 °C at Farol Island, Brazil, Oliveira and Masunari 1995 and 20.8 – 28.7 °C at Paranapuã Beach, Brazil, Micheletti-Flores and Negreiros-Fransozo 1999). In Brazil the highest densities of *P. armatus* occurred during summer when mean surface water temperatures were > 25 °C (Oliveira and Masunari 1995). Upper thermal tolerance has been shown to be correlated with surface water temperatures and maximal temperatures of microhabitats (Stillman and Somero 2000).

P. armatus can likely survive increased temperatures as a result of low tide exposure as long as microhabitats remain below this upper thermal limit.

During the winter in Georgia, water temperatures are well below the normal minimal temperature of the crab's native range (< 16 °C). This seasonal drop in temperature may expose crabs to temperatures below their lower thermal limit, thus causing the decrease in densities we observed. This effect was documented for blue crabs, *Callinectes sapidus*, at Sapelo Island when water temperatures fell below 15 ° C (Fitz and Weigert 1991). Seasonality has also been shown to affect the strata habitation of mud crabs (*P. herbstii* and *E. depressus*) in oyster reefs of North Carolina. During warmer months crabs more frequently inhabited the cluster stratum of the oyster reefs, whereas in the colder months they inhabited the subsurface stratum (Meyer 1994).

Salinity Gradients

In addition to thermal patterns, salinity generally increases from the Upper regions of the estuary to the Lower regions where the river meets the sound (Bahr and Lanier 1981). Salinity data collected from the SINNER hydrographic stations during 2003, show salinities for the warmer months (June – September) to be quite similar for the Upper region (~ 15 – 27.5 ppt) and Lower region (~ 13 – 27.5 ppt). By October (and continuing through December), there was a narrowing of the salinity ranges for both regions, and they remained quite similar (Upper: ~ 23 – 29 ppt, Lower: ~ 22 – 30 ppt). The salinities documented for the Duplin River were well-within the range for *P. armatus* in southern Brazil. Oliveira and Masunari (1995) found an annual salinity range of 6.7 – 31.5 ppt,

and Micheletti-Flores and Negreiros-Fransozo (1999) found a range of 19.3 – 34.0 ppt. Shifts in seasonal precipitation patterns and their resulting shifts in salinity of estuarine waters may not be as strong a force as thermal tolerance and exposure in determining estuarine-wide porcelain crab dynamics.

Reproduction

The dramatic seasonal change in densities, the considerable recruit densities in warmer months (Figures 4 and 5), the ability of females to reproduce as small as 3 mm CW, the high percentage of ovigerous females (Figures 6 and 7), and the high densities of *P. armatus* zooea we noted in plankton tows during the summers of 2003 and 2005 (A. Hollebone and W. Morrison *personal observation*) all attest to the remarkable ability of the crab to rapidly increase from tens to hundreds of crabs m^{-2} in the winter to thousands and tens of thousands of crabs m^{-2} in the summer.

Although the sex ratio of *P. armatus* sampled in both estuaries varied considerably (Table 1), there were no consistent or statistically significant patterns for males or females dominating in any region, tidal height, or season. An overall annual sex ratio for *P. armatus* at Farol Island, Brazil was ~ 1.05 (males : females) (Oliveira and Masunari, 1995). Our data were consistent with this figure.

It is interesting to note the proportions of the sexually mature (≥ 3 mm CW) female population that were ovigerous during the warmer months. At the Duplin River sites we found a range of ~ 20 – 85 % and for the Wilmington River sites a range of ~ 20 – 90 %

throughout the year. Comparatively, Oliveira and Masunari (1995) found that 47.8 % of all females ≥ 4 mm CW (33.7 % ≥ 3 mm CW) collected throughout their study were gravid (data from all seasons). This figure falls within the range for both Georgian estuaries as well as an overall proportion calculated for all females ≥ 3 mm CW at all locations throughout the year (Duplin River: 30.5%; Wilmington River: 48.1%).

What appears to have shifted with the crab's northward progression is the size at which females mature. Oliveira and Masunari (1995) noted that females matured at 4 – 4.9 mm CW, while we found mature females at 3 – 3.9 mm CW. Thus, not only are many females gravid at any given time of the year, but there are more total females that can reproduce. They appear to become sexually mature at a smaller size, potentially increasing the number of broods produced per year or the number of times they brood in a lifetime.

In both rivers we found that the proportion of gravid females was highest during early and late summer in the Upper regions of the estuary. We also found that adult crabs can survive through the colder months of the year. Therefore, these data suggest that *P. armatus* may be capable of sustaining its populations locally, rather than relying on regional inputs of propagules during the warmer months of the year.

Biological Factors

Introduced species often have large impacts on assemblages of native species via predation and competition for resources (e.g., Nichols *et al.* 1990, Lodge and Kershner

1994, MacIsaac 1996), and native species may repel invasions of non-indigenous species via the same interactions (e.g., Stachowicz *et al.* 1999, Parker and Hay *in press*). Therefore, in addition to the abiotic factors, the population dynamics of *P. armatus*, could be affected by interactions with native crabs.

Although this study was not intended to experimentally test the impacts of competition and predation on community structure, we correlatively addressed this possibility by evaluating how crab density for both native mud crabs and non-native porcelain crabs covaried over space and time. Out of the 48 possible correlations of mud crabs versus porcelain crabs in both estuaries across all region-tidal height combinations and seasons, we saw significant positive relationships in 23 % of the contrasts. Significant negative relationships never occurred. Positive correlations occurred during warmer months when *P. armatus* densities were increasing and those of mud crabs were at their peak. These patterns are correlations rather than experiments, but lend no support to the hypothesis that the invasive crab is negatively affecting the native crabs with which it most closely associates.

Impacts

Although widespread impacts on oyster reefs of the invasion are not apparent at first glance, the crabs' high densities alone suggest that changes in these communities may be occurring. Our initial efforts to tease apart any impacts that the crab may be having were performed in a mesocosm setting and were further tested in multiple field experiments (this aspect of the research will be developed further in A. Hollebone's doctoral

dissertation). At the smaller, experimental scale we observed some changes in the composition of the reef community (e.g., depressed oyster growth, microalgal moderation, macroalgal facilitation) over relatively short periods of time, suggestions of what we may find at the larger scale.

Native Species Richness

Biological invasions are considered to be one of the leading causes of a growing global biodiversity crisis by directly or indirectly altering local community composition and diversity (Stachowicz *et al.* 2002 and references therein). Shifts or losses in native species richness are indicators by which we can determine the effect of a biological invader on affected communities. In our mesocosm experiment, we tracked the recruitment of native species into areas with and without porcelain crabs. The density at which we seeded our experimental baskets (1500 crabs m^{-2}) did not elicit any effect on such recruitment. We only observed a significant increase in richness across all baskets over time. In comparison with our monitoring data (Figures 2 and 3), the density of porcelain crabs seeded in our experimental units was appropriate and actually elicited significant responses for oyster and algal growth in the mesocosms as well as oyster growth, juvenile oyster settlement, and predation shifts in field experiments (A. Hollebone *PhD. dissertation*). Although these data did not suggest any impacts that *P. armatus* may be having on reef community richness, we took a more focused look at any changes in species-specific phenomena.

Oyster and Mussel Growth and Survivorship

As a result of overharvesting and increasing destructive pressures on coastal habitats, oyster reefs of the southeastern U.S. have experienced large decreases in their extent and health over the past few decades (Lenihan and Peterson 1998 and references therein). With the introduction of *P. armatus* at high densities in these habitats, we questioned their impact on the growth and survivorship of oysters. Through our mesocosm work, we could select oysters of a smaller initial size (< 60 mm long) and measure their growth in the presence or absence of porcelain crabs over time. We found that the crabs led to significantly depressed growth within the first 4 weeks of exposure. Survivorship (those oysters that were alive and still labeled) upon termination of the experiment (12 weeks) was quite low (~ 16 – 21.5%) but not significantly different between treatments. These data suggest that porcelain crabs may be leading to smaller oysters in the long term. We have two possible explanations for this development: (1) oysters and porcelain crabs are both filter feeders and are, therefore, competing for a common food resource, and/or (2) the close association of the crabs to oyster surfaces may actually be scaring the oysters, causing them to close their shells and not feed. This same effect was further supported by field experimentation in which the presence of porcelain crabs (1200 crabs m^{-2}) led to a significant depression in oyster growth (t test, $p = 0.040$, A. Hollebone *PhD. dissertation*).

On the other hand, mussels did not appear to be affected by the presence of *P. armatus* in the same fashion as oysters. They survived relatively well (~ 69 – 74%) and grew ~ 2.5 mm in both treatments. Perhaps mussels utilize different classes of food particles or are more tolerant of the movements of other oyster reef-dwelling organisms.

Microalgal Biomass

An interesting effect of porcelain crab presence on oyster reef communities was the apparent moderation of microalgal growth on oyster surfaces in the face of seasonal fluctuations. As chl *a* measurements taken from areas without porcelain crabs increased dramatically early in the study and then fell below initial concentrations towards the end of the study, microalgal biomass in areas seeded with porcelain crabs appeared to be moderated, leveling out at a fairly consistent 6-8 $\mu g\ cm^{-2}$. These results may be explained by the close physical association of the crabs with the oyster surfaces. The movements of the crabs over the surfaces of the oysters may be removing the build-up of sediments and particles settling out of the highly turbid waters of the SAB. In addition, porcelain crabs may be clipping or scraping small alga growing on oyster surfaces as additional food sources (A Hollebone *personal observation*, Gabaldon 1979 and Kropp 1981: alternate feeding methods).

Macroalgal Growth

Despite the apparent moderation of microalgal biomass by porcelain crabs, the growth of large macrophytes (seaweeds) appeared to be facilitated by the presence of the crab. Within 8 weeks, 60 - 65% of all oyster surfaces were covered by *Ulva spp.* and/or *Enteromorpha spp.* when in the presence of *P. armatus*. In other marine systems, algal biomass has also been shown to increase with the introduction of an exotic (Pelegri and Blackburn 1995, Kotta *et al.* 2001). These results have been attributed to increased biodeposition or bioturbation rates. Because there are almost 7 X as many porcelain

crabs than native mud crabs in oyster reefs during the warmer months of the year, increased activity around oysters may be clearing off substrates onto which algal spores settle and may be re-working and suspending nutrients that support algal growth. The growth of large macrophytes to this extent has not been apparent in the field and may be under the control of grazing by herbivores (e.g., fish and crabs) that can access oyster reefs in the field but were not incorporated in our mesocosm experiment.

Summary

Since its first documentation in oyster reefs of the South Atlantic Bight in 1995, *Petrolisthes armatus* has increased from a single individual m^{-2} to mean summer densities of several thousand individuals m^{-2}. Despite these high densities and widespread distribution throughout estuaries, there have been no obvious dramatic impacts of the crabs on native communities to date. We can speculate on a number of reasons why we have not seen any changes. Unlike recent introductions of predatory crab species that have drastically altered the activities of individual species (Trussell and Nicklin 2002) or "reorganized" native and previously invaded assemblages (Lohrer and Whitlach 2002), *Petrolisthes armatus* is a filter feeder removing particulates from the water that passes by their habitat versus consuming what is directly in their habitat. The crab may actually be filling an open ecological niche, and, thus, does not have any profound effects on the native species already present. On the other hand, the invasion of *P. armatus* may be too recent for mounting impacts to be obvious, or we may not have investigated the crab enough or for long enough to notice their impacts at a large scale.

There is also the possibility that the northward movement of *P. armatus* and its drastic increase in density is due to increasing water temperatures (temperatures suitable for a historically tropical species) as an effect of global climate change. Previous studies have documented similar shifts for tropical fishes on temperate reefs off of North Carolina (Parker and Dixon 1998) and more southern species of invertebrates in the rocky intertidal habitats of California (Barry *et al.* 1995). Although we cannot make a certain connection between global warming and regional shifts in porcelain crab ranges, the crab is clearly finding new habitats that fit its needs and numbers are increasing dramatically over populations in native habitats. Although we currently have experimental indicators of what may be affected by the invasion, the long-term, large-scale ecological effects of *P. armatus* on native communities have yet to be seen .

ADDITIONAL RESEARCH

In addition to the research described above, we also performed a series of field experiments to elucidate how community/habitat composition and native species function influence the success of the porcelain crab invasion and to assess the impacts of the crabs on oyster communities in the field. Although this work was not explicitly developed in the original fellowship proposal or addressed in this report, it will be extensively covered in the doctoral dissertation to follow in the coming months.

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PUBLICATIONS

Currently, we have three manuscripts in preparation for submission to peer-reviewed journals.

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